STUDIES OF AN IMPLANTED MURINE MYELOGENOUS LEUKEMIA C1498[1]

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This study described changes in the characteristics of a transplantable murine myelogenous leukemia (C1498) since its initial isolation in 1941. Demonstration of C-type "virus" particles, ability to maintain the disease as an ascites tumor with single cell preparations and ability to cause development of the disease by injection of peripheral blood leukocytes support the original identification of the disease as a leukemia. Characteristic anemia was seen without a corresponding steady granulocytosis. The leukocyte counts exhibited cycling and at least two distinct causes of death were suspected. The tumor is surrounded by a fibrous capsule suggesting a rejection phenomenon. Large numbers of morphologically mature granulocytes were found in the blood throughout the disease course, although the number of immature forms increased steadily as the disease progressed. Granulocytic infiltration of the spleen and liver was evident and growth of the tumor when transplated under aseptic conditions was certain. It is suggested that the recipient mice showed increased recognition of tumor cells because inflammatory and immune responses to the invading cells increased. It appears that the characteristics of a true myelogenous leukemia are masked by the presence of an inflammatory response in the host C571B1/6J mouse strain.

On September 5, 1975, Jim Graham succumbed to the disease he so diligently investigated.

The changed disease course and pathology of C1498 murine myelogenous leukemia are of particular interest for a variety of reasons. It was shown that "C-type" particles can be found in the subcutaneous tumor occurring after transplant of the leukemic cells, although a causal relationship has not been demonstrated (Graham and Crang, 1971). The C1498 leukemia has also been used in the study of the effects of granulopoietic control substances on murine myelogenous leukemia (McMahon, 1972; Graham and McMahon, 1973; Graham, 1973). Although lymphocytic leukemias are common in rodents, the C1498 variety is one of only five rodent myelogenous leukemias (compared with 17 lymphogenous) described in the compendium of transplantable tumors by Dunham and Stewart (1953). It should be added that recently, virally-induced myelogenous leukemias have been reported, including the isolation of a myelogenous leukemia-inducing virus (MyLV) from a chloroleukemia cell line originally induced in C57B1 mice by the Friend virus complex (McGarry, *et al.*, 1974).

The tumor we studied was in approximately its 1,225th passage when brought to Bowling Green after original identification by J. Brandenberg at the University of California in 1941, and transferred to Jackson Laboratory in 1949 (Jackson, 1968). Since 1970, the tumor has been passaged more than 50 times in our laboratory. The gross appearance of the tumor has remained unchanged (Dunham and Stewart, 1953; Jackson, 1968), although marked differences in the disease course and hematologic symptoms were found. Tumor cells were transplanted into the axillary region of the host mouse by trochar with 100 percent takes producing a palpable tumor 5–7 days after implantation. It appears as a mass of dark red hemorrhagic tissue closely attached to the skin.

The primary hematologic symptom of the C1498 leukemia was the observation of lack of granulocytic differentiation (Jackson, 1968). We observed major

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changes in the hemotologic response to implantation of the tumor cells. The most startling was the high degree of granulocytic maturation found, including large numbers of fully segmented neutrophils. Other significant changes in the disease course and in histologic observations may explain the dramatic differences found in hematologic values.

METHODS

The tumor used in this study was the myelogenous leukemia C1498, which is carried in the C57B1/6J inbred mouse strain. Both the tumor and the host mice were originally obtained from the Jackson Laboratory, Bar Harbor, Maine.

The 10-day-old tumor bearing mice were sacrificed, and the tumors were removed and finely minced in sterile saline. Cell viability of the minced tumor was determined by the trypan blue dye exclusion method and was always found to be greater than 99%. The minced tumor-saline solution was injected aseptically by trochar into the axillary region of the recipient mice as a subcutaneous inoculation. No signs of ill effects or infection arising from the inoculation were seen. The tumor-bearing mice were kept in constant temperature and humidity in an environmental chamber, and provided with food and water *ad libitum*. In all experiments, male and female mice six weeks of age and older were selected at random from the breeding colony.

Blood was taken from the tail vain and collected in a small vial containing 0.2 mg/ml blood of ethylene diamine tetraacetic acid, disodium salt (EDTA). Total erythrocyte and leukocyte counts were done on a Model B, Coulter Counter (Graham and Morrison, 1970). Additional blood was obtained from the tail vein, placed in a heparinized capillary tube and centrifuged at 13,500 RPM for three minutes to obtain the packed cell volume (hematocrit). Leukemic animals were sampled on the sixth, eighth, twelfth and eighteenth days post-implantation. Normal mice were sampled on the same day the tumor was implanted into the experimental mice. Twenty animals were used at each of these sampling points.

Peripheral blood differential counts were made from smears stained with Jenner-Giemsa (Morrison, 1967). Identification of granulocytes was done according to the compartment concept of Boggs, *et al.* (1965). Classification of the main groups of the erythroid, lymphoid and other cell types was based on Harris and Burke (1957) and Morrison (1967).

Spleen, thymus, liver and tumor tissues were obtained immediately after natural death of the leukemic mice and fixed in a formalin-acetic alcohol fixative for 24 hours. Tissues were drained and placed in automated butyl alcohol-dehydration and paraffin-infiltration series. The individual dehydrated and infiltrated tissues were embedded in 56°C Paraplast (Sherwood Medical Industries, Inc.). The blocks were cooled and cut into 8–10 micron sections and placed on slides coated with gelatin adhesive. The finished slides were dried for 2–3 hours on a heating plate at 43°C before staining with hematoxylin and eosin. A one-way analysis of variance with repeated measures was done by computer to measure the significance of the erythrocyte, hematocrit, total leukocyte and differential leukocyte counts.

RESULTS

The time of death of nearly 200 untreated leukemic mice are presented in figure 1. The number of mice that died on each day of post-tumor implantation resulted in bi-modal distribution rather than a bell shaped curve. The erythrocyte counts for untreated leukemic mice dropped throughout the course of the disease (fig. 2, left). It should be noted that the largest drop occurred between the 8th and 12th days. The range of values at each sampling day are shown with the superimposed black bar and it is evident that the range of the counts broadened with the passage of time. The hematocrit values (fig. 2, center) showed the same pattern as the erythrocyte counts with the range of values at a particular sampling point increased from 0 to 18 days.

The total leukocyte counts for untreated leukemic mice are shown in figure 2, right. The average count did not increase consistently as the disease progressed. In fact, the average count increased by 6 days post-injection, dropped at 8 days and again rose at 12 days. It should also be noted that the ranges were in all cases very large, particularly at 6 and 12 days.

The percentage and absolute cell counts for early granulocytes seen in figure 3, top, showed the same pattern as the total leukocyte count with low values. The intermediate granulocytes followed this same trend (fig. 3, center). The absolute cell count for the mature granulocytes showed a consistent increase from 0 to 18 days, while the percentage of mature granulocytes at 6 and 8 days was lower than that at 0 days (fig. 3, bottom).

The percentage of eosinophils greatly decreased at all four post-implantation sampling points when compared to the inital days value. The only significant change in the absolute cell count of eosinophils was the four-fold increase at

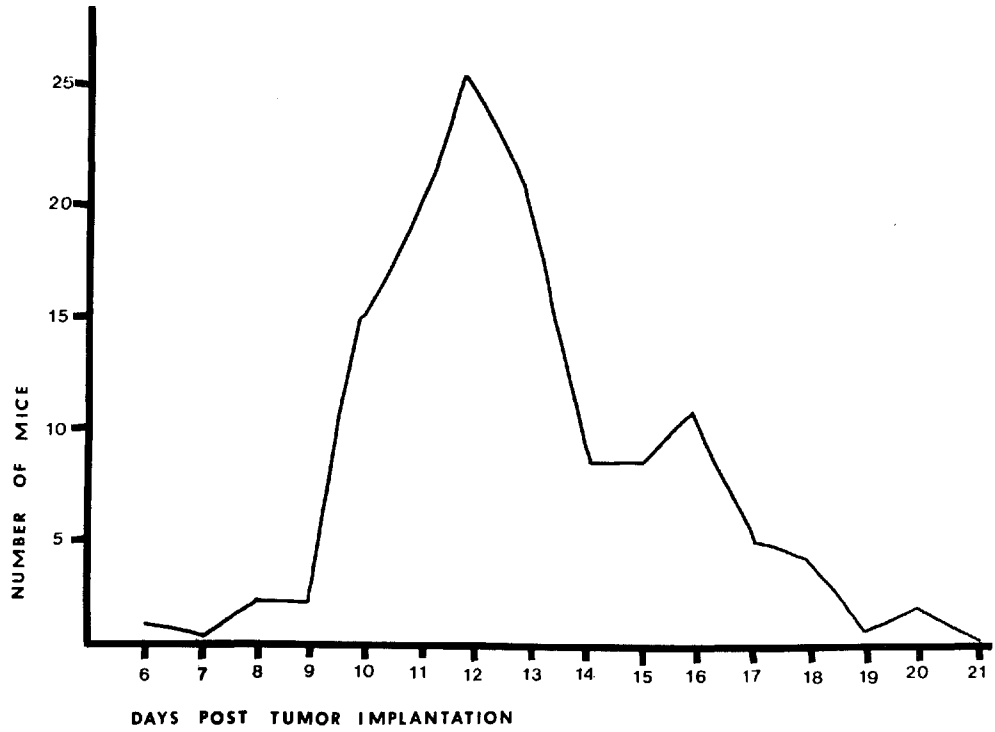

FIGURE 1. Time of death of mice implanted with the C1498 leukemia tumor. The bimodal distribution differs from the expected bell-shaped curve.

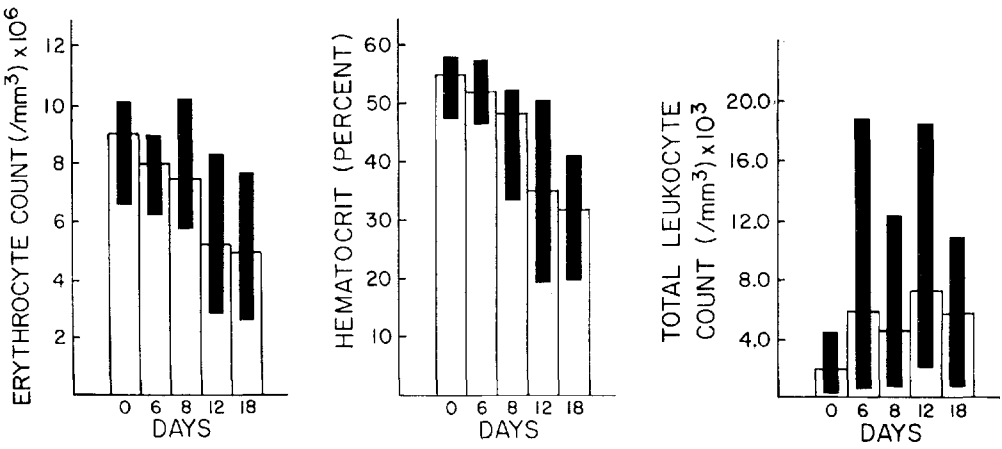

FIGURE 2. Erythrocyte, hematocrit and total leukocyte values (left to right) for leukemic mice at 6, 8, 12 and 18 days post-tumor implantation.

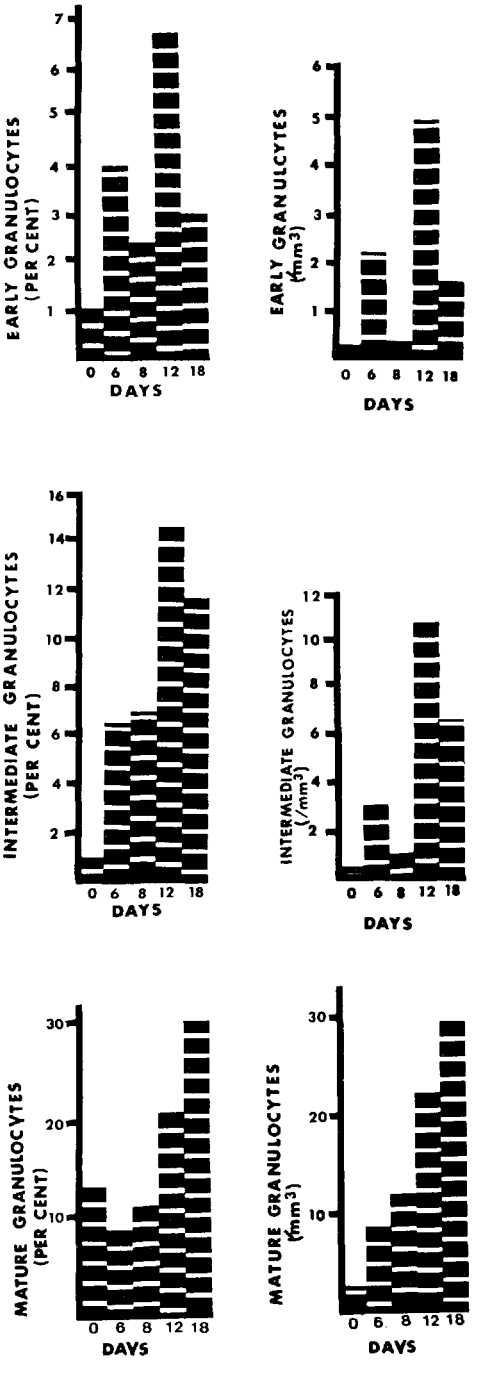

FIGURE 3. Percentage and absolute number of early, intermediate, and mature granulocytes (top-bottom) in the blood of leukemic mice after tumor implantation.

12 days (fig. 4, top). The percentage of lymphocytes dropped drastically (to one half) from 0 to 6 days and stayed at this low level through to the eighteenth day. The absolute cell count of lymphocytes increased significantly at 6, 8 and 12 days post-tumor implantation (fig. 4, bottom).

The differential leukocyte counts found at each of the four sampling points (6, 8, 12 and 18 days post-tumor implantation)

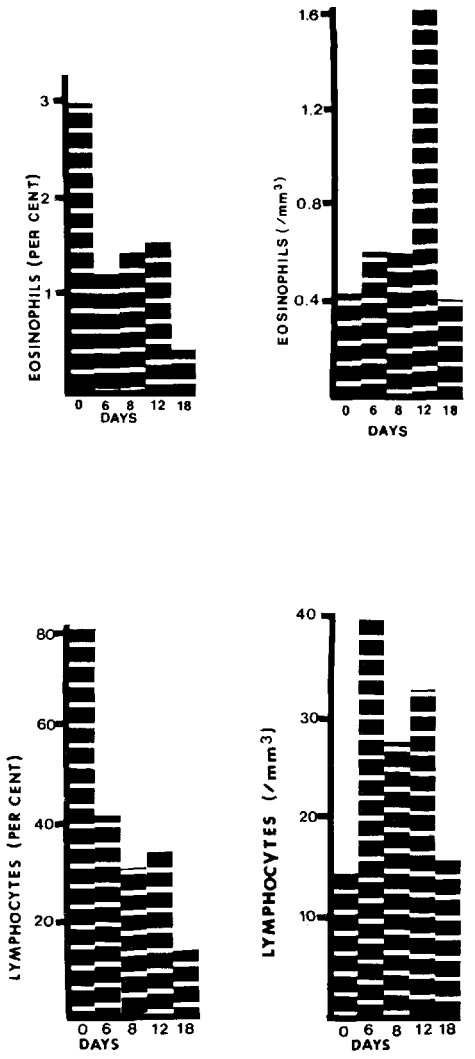

FIGURE 4. Percentage and absolute number of eosinophils (top) and lymphocytes (bottom) in the blood of leukemic mice after tumor implantation.

were compared with standard values obtained from the literature (table 1). Peripheral counts are extremely variable in the leukemic mice and it was impossible to demonstrate statistically significant changes in numbers of the various cell types. The total granulocyte values of 25% at 6 days, 37% at 8 days, 41% at 12 days, and 69% at 18 days suggest an increasing granulocytosis, when compared with the normals of 28% to 28.5% reported by Scarborough (1931) and Petri (1933). Examination of peripheral blood films revealed a large number of morphologically mature segmented neutrophils (50% at 18 days) as well as relatively fewer (15.5%) metamyelocytes and ring or stab forms. The cells did not differ from neutrophils obtained from normal mice.

at the time of death of the mice was also seen. Granulocytic cells were seen surrounding the blood vessels of the liver. Degree of involvement as measured by the relative number of granulocytic cells surrounding the capillaries varied but was found in nearly all mice by the time of death. Splenomegaly was frequently evident but rarely exceeded a 50% increase over the average spleen weight of control mice (0.06 gms). Some invasion of the red pulp of the spleen was regularly seen and the distinctiveness of the red and white pulp was blurred by the granulocytic infiltration. The splenic capsule was often also invaded by granulocytic cells. No significant involvement of the thymus was apparent.

The results of this study demonstrate the complexities involved in the use of

TABLE 1

Comparison of peripheral leukocyte counts from leukemic and normal mice.

Cell Type	% Normal Value* From Literature	Experimental**			
		Leukemic 6 day	Leukemic 8 day	Leukemic 12 day	Leukemic 18 day
Early Granulocyte	——	3.9	2.4	6.8	2.9
Int. Granulocyte	——	5.4	6.9	10.5	15.5
Mature Granulocyte	——	15.1	26.3	22.1	50.1
Eosinophil	2.0	1.0	1.0	2.0	1.0
TOTAL GRANULOCYTES	28	25.4	36.6	41.4	69.5
Lymphocytes	65–68	73.0	63.0	45.0	30.0
Monocytes	7–7.5	1.0	1.0	1.0	1.0

*Scarborough (1931), Petri (1933), as reported in Wintrobe, *Clinical Hemology* (1967).
**Percentage of differential leucocyte counts.

DISCUSSION

The histologic appearance of the subcutaneous tumor arising from implantation of minced tumor into a normal host appeared as a highly vacuolated mass of rounded cells of variable size surrounded by a dense connective tissue capsule. Limited vascularization was apparent and appeared distinct from the large number of vacuoles. In previous studies, the density of the tumor resulting from the close packing of the cells appeared to increase progressively throughout the disease course (McMahon, 1972). The vacuoles may represent localized necrosis.

Leukemic involvement of other organs

murine myelogenous leukemia as a tool in the study of hematologic neoplasias. Both the hematologic data and the pathologic studies indicate that the symptoms of leukemia are interwoven with a marked inflammatory response by the host mouse.

The erythrocyte and hematocrit counts showed a consistent decrease as the disease progressed (fig. 2, left and center). The red cell decrease was not gradual or constant, however and the largest drop occurred between the eighth- and twelfth-day post-implantation. There was only a small decrease in the erythrocyte count from the twelfth to the eighteenth day.

This is evidence that the fatigue and weakness caused by anemia reaches its peak near the twelfth day. It may be noted in both figures that the range of counts at a particular sampling time increased progressively from 0 to 18 days post-implantation.

The total leukocyte counts (fig. 2, right), did not show a steady increase in the number of cells as might be expected in leukemia. Instead the counts at 6 days increased 50 percent over the control counts. Leukocyte count decreased at the 8-day sampling point, and rose to its peak at 12 days, only to decrease by the eighteenth day. Cycling is possible explanation for these readings. Cycling is a phenomenon that has repeatedly been observed in cases of leukemia. The symptoms for each individual vary widely and intermittent remissions of the disease have been frequently recorded. Although cycling has not been previously documented in studies of murine leukemia, it has been known in human leukemia for many years. Wintrobe (1967) refers to a case where the leukocyte count ranged from extreme leukocytosis to leukopenia in three cycles in a period of 30 months.

Besides the cycling, which was evident in the peripheral blood counts, two major causes of death are postulated from our study of the number of deaths on each of the days post-implantation (fig. 1). Note that the number of deaths did not follow the characteristic unimodal bell curve. Instead two modes were found, one at 12 days and one at 16 days. Coupled with this was the observation that nearly all of the mice dying on or before the thirteenth day were comparatively healthy. In these mice a tumor was usually evident, but the fur was shiny and they were generally active. The mice that survived to the sixteenth day and beyond were puffy, had noticeable infections, showed little activity and generally appeared very sickly. Comparing survival data with the observed erythrocyte, hematocrit and leukocyte counts, it appeared that the deaths which occur at the twelfth-day day peak were primarily attributable to anemia. Those mice which had sufficient red cells to survive this crisis, succumbed to secondary infection or failure of a vital organ due to infiltration by leukemic cells.

Cycling was evident in the differential white cell counts where the largest numbers of immature granulocytes (both early and intermediate) occurred at 6 and 12 days with a moderate elevation at 18 days (fig. 3, top and center). An interesting, and rather unexpected, trend was the elevation of mature granulocytes in the blood throughout the course of the disease (fig. 3, bottom). It appears that the difficulty was not so much that there are too few mature granulocytes to meet the demands of the individual but that the presence of additional large numbers of immature granulocytes disrupted proper function of the phagocytic and, perhaps, immune mechanisms. The large number of mature granulocytes may have resulted from the marrow storage compartment pouring cells into the peripheral blood in response to the abnormally high percentage of immature granulocytes and the onset of infection, or to increased granulopoiesis which is suggested by the timing of the increase. The percentage of lymphocytes dropped markedly as would be expected with the onset of myeloid leukemia, however, the absolute cell count of lymphocytes was increased over the value for normal mice at 6, 8 and 12 days (fig. 4, bottom). The only significant increase in the absolute count of eosinophils was at 12 days (fig. 4, top).

The fibrous capsule surrounding the tumor and the low vascularity of the tumor tissue coupled with intense vacuolation suggest that the host animal walled off the "foreign" tissue with an intense rejection response. This was apparent, although the transplanted tumor cells came from isologous and highly inbred C57B1/6J mice raised under identical conditions in our laboratory for three years. The walling off of the tumor tissue accompanied the marked neutrophilia evident in the blood (table 1), suggesting that the unusual characteristics of the C1498 myelogenous leukemia may have been due to the host's response to the tumor implant rather than a true leukemia. This possibility is enhanced by the high level of mature

granulocytes (Table 1) found in the "leukemic" animals. The increase in immature granulocytes formed progressively during the disease course may have been due to the increased granulopoiesis taking place in the bone marrow. This would appear to be a classic "shift-to-the-left" normally seen in the response to acute infection.

Granulocyte infiltration in the spleen and liver was present as expected in any massive response to infection as well as in a true myelogenous leukemia but no thymic involvement was seen. Possibly the recipient C57B1/6J mice had an increased recognition of the tumor cells as "foreign" and hence, granulocytic and immune response to the invading cells increased.

The demonstration of "C-type particles," the ability to maintain the "disease" as an ascites tumor by intraperitoneal injection, and the ability to cause the development of the leukemia by injection of cells isolated from peripheral blood all support the original identification of the C1498 transplantable tumor as a leukemia. It is suggested that the characteristics of a true myelogenous leukemia are masked by the presence of a massive neutrophilic inflammatory response on the part of the host mouse.

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